STUDIES ON THE NUTRITIONAL VALUE OF SOYBEAN FLOUR TO TRIBOLIUM CONFUSUM DUV.[1,2]

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It is generally stated that the confused flour beetle, *Tribolium confusum*, can attack peas and beans as well as grains and many other things (Good 1936). There is, however, little information regarding this insect as a pest of soybean products. Mickel & Standish (1946) in studying the susceptibility of edible soya products to attack by *T. confusum* found that this insect could not develop normally on their samples of soy flours and soy grits. The rate of larval development was so greatly decreased that serious infestation seemed unlikely. The reasons for the deficiences of soy as a food for this beetle were not determined by these authors. It is the purpose of the present paper to report some studies trying to account for the unsuitability of one particular soybean flour as food for this insect.

MATERIALS AND METHODS

A defatted soybean flour, Nutrisoy R, supplied through the courtesy of Dr. J. W. Hayward of the Archer-Daniels-Midland Company, was used in all the experiments.[3] The flour was light

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[2] A more preliminary report on this work was submitted by S. L. in partial fulfillment of the requirements for a Master of Science degree. Both authors would like to acknowledge their appreciation to Dr. Gottfried Fraenkel for critically reading the manuscript.

[3] The defatting during manufacture was performed by extraction with hexane. An analysis of this particular sample was made for us by chemists at the Archer-Daniels-Midland Company. This analysis gave: moisture 7.29%, protein 52.76%, water soluble nitrogen 46.8%, fat 0.96%, fiber 2.79%, ash 5.73%, calcium 0.44%, phosphorus 0.67%, urease 0.30%, choline 0.222%, thiamin 3.41 parts per million, riboflavin 4.36 p.p.m., carotene 3.04 p.p.m. The analysis was made when the experiments were begun (August 1948); the sample was then used for experiments extending over a period of somewhat more than two years.

yellow in color, very fine, rather powdery and free flowing. It could pass a 100-mesh wire screen readily, and the particles did not stick together to form lumps.

The soybean flour was used alone: either extracted,[4] or autoclaved,[5] or untreated; it was also tested in combinations with yeast,[6] or with whole wheat flour,[7] or with sterol and the B vitamins[8] reported to be required by the insect under study (Fraenkel & Blewett 1942–1947). Whole wheat flour alone, yeast alone, and whole wheat flour plus yeast were employed as check diets.

The stock culture of beetles was maintained at about 26° C. and 70 per cent R.H. on whole wheat flour plus 5 per cent dried yeast. Eggs were sifted from the medium and hatched at 30° C. and 70 per cent R.H. For all tests newly hatched larvæ (< 24 hours old) were used.

Both individual and mass-rearing methods were used. For mass-rearing tests, newly hatched larvæ were tested in lots of 25 on the various diets. Rearing was done mostly in flat stender dishes (4.5 × 2 cm.) which were found to be satisfactory for preventing escape even when less palatable foods were used. In earlier experiments 1.5 gram, and in later ones 4 grams, of each diet were used per replicate. These amounts were more than adequate because preliminary tests indicated that only about 10 mg. of food were consumed by a single larva. Four to nine replicates were made for each diet. For individual rearing tests, newly hatched larvæ were introduced into short vials (1.6 × 2 cm.) in which 0.1 or 0.2 gram of food material had been placed.

[4] In a Soxhlet apparatus for two hours with a mixture of ethyl ether and absolute ethyl alcohol in the proportion of 2.5: 1 by volume. The extracting solvent was later driven off by drying in a vacuum oven at 65° C. for a day or two.

[5] At 15 lbs. for 20 minutes.

[6] Strain G primary dried yeast prepared by the Anheuser-Busch, Inc.

[7] Passed through a 60-mesh wire screen which removed most of the bran material.

[8] The sterol and the B vitamins used were C.P. preparations. The amounts used per gram of diet were as follows: thiamin hydrochloride 4 μg., riboflavin 6 μg., niacinamide 32 μg., pyridoxine 4 μg., calcium pantothenate 16 μg., folic acid 0.8 μg., biotin 0.05 μg., choline chloride 0.5 mg., *p*-aminobenzoic acid 0.5 mg., inositol 0.5 mg., and cholesterol 10 mg. The B vitamins were dissolved in a small quantity of distilled water, added to the diet, and then well mixed.

The dishes or vials containing the newly hatched larvæ plus food were placed in glass containers in which a R.H. of 70 per cent was maintained by means of a sulfuric acid solution prepared according to Buxton & Mellanby (1934). No attempt was made to check the humidity within the containers but the acid solution was replaced occasionally with fresh solution to assure approximate constancy. Neither the containers nor the insects were sterilized but no visible amount of mold develops at this humidity. After several months the flour does become lumpy.

The insects were left to grow for the first two weeks undisturbed; frequent inspections were made thereafter to look for pupæ. The pupæ were removed as soon as they were found and stored immediately in an oven at about 55° C. until all the pupæ in a lot had been collected. Then the pupæ were dried at 105–110° C. overnight and again for 1 hour periods until constant weight was obtained. From the group value, the dry weight of an individual pupa in the lot was calculated by averaging. The weights given in the table are the means of the weights so calculated for all the lots.

The adequacy of the different flour preparations were compared on the basis of pupal weight, percentage of the insects that pupated, and duration of the larval stage. The data were subjected to the analysis of variance, and the significance of the difference of the means is based at the 5 per cent level. In no case was variation between different replicates of a single treatment found to be significant.

RESULTS

Most of the results are summarized in Table 1 and presented graphically in Figures 1 and 2. The results obtained from mass and individual rearing corresponded very closely, although in general the individual rearings resulted in somewhat higher pupal weight, higher percentage of pupation, and shorter larval period (individual rearing experiments were based on twenty specimens per curve).

In Table 1 and Figure 1 it is shown that on Nutrisoy flour alone, the growth of the confused flour beetle was very much delayed. In terms of pupal weight, percentage of pupation, larval

TABLE I

PUPAL WEIGHTS, PERCENTAGES OF PUPATION, AND LARVAL DURATIONS OF TRIBOLIUM CONFUSUM GROWN ON VARIOUS DIETS REPRESENTED IN FIGURES 1 AND 2

Diet	Pupal Weight (mg., dry)		Per Cent Pupation		Larval Duration (days)	
	Fig. 1	Fig. 2	Fig. 1	Fig. 2	Fig. 1	Fig. 2
Nutrisoy R alone	0.63[1]	0.66	79.6	82	51.5	51.0
Nutrisoy R + whole wheat flour (3:1)	0.75	.....	95.6	.....	24.1	.....
Nutrisoy R + whole wheat flour (1:1)	0.79	.....	89.3	.....	22.3	.....
Nutrisoy R + yeast (10:1)	0.85	.....	97.3	.....	21.7	.....
Nutrisoy R + B vitamins	.....	0.72	.....	80	.....	42.0
Nutrisoy R + cholesterol	.....	0.84	.....	92	.....	32.3
Nutrisoy R + choselterol + B vitamins	.....	0.92	.....	98	.....	29.5
Autoclaved *Nutrisoy R*	.....	0.66	.....	80	.....	59.4
Extracted *Nutrisoy R*	—	0.38[2]	0.0	1[2]	—	106. [2]
Extracted *Nutrisoy R* + extract of *Nutrisoy R*	0.69	.....	55.1	.....	65.0	.....
Extracted *Nutrisoy R* + yeast (10:1)	0.84	.....	97.8	.....	21.7	.....
Extracted *Nutrisoy R* + cholesterol	.....	0.85	.....	90	.....	37.9
Extracted *Nutrisoy R* + cholesterol + B. vitamins	.....	0.87	.....	95	.....	32.0
Whole wheat flour	0.83	.....	96.4	.....	19.8	.....
Whole wheat flour + yeast (10:1)	0.99	1.03	99.6	99	18.2	17.9
Yeast alone	0.88	.....	98.2	.....	21.9	.....
Significant difference at:						
5 per cent level	0.04	0.06	6.4	9	3.5	3.8
1 per cent level	0.06	0.09	8.6	12	4.7	5.2

[1] Weight of late pupæ only. These were appreciably smaller than early ones which were inadvertently overheated and ruined.

[2] The lone pupa obtained on extracted *Nutrisoy R* was presumably made possible by the growth of microorganisms. At least, the powdery flour was getting lumpy after such long standing in the container.

duration and spread of pupation time, Nutrisoy is clearly much inferior to whole wheat flour, and more so to whole wheat flour plus yeast.

When whole wheat flour was added to the Nutrisoy, the food value of the latter was considerably increased. Actually, the curves for 3:1 and 1:1 mixtures cross (Fig. 1), but the differ-

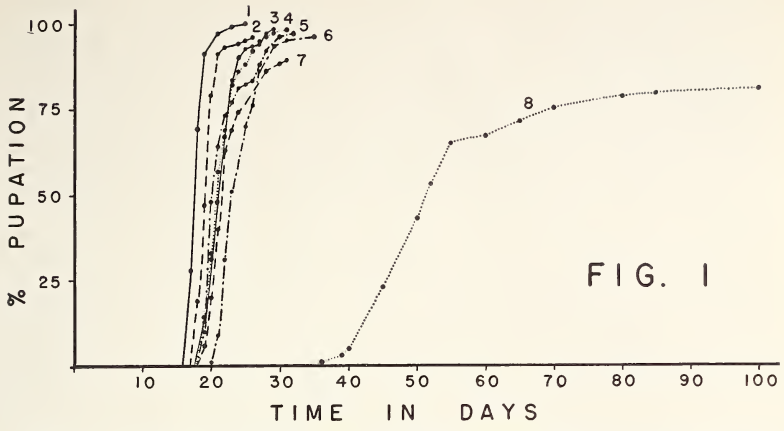

FIG. 1. Growth curves for larvæ of *Tribolium confusum* reared on various diets. Each curve based on data from 225 individuals. Curve no. 1 is for whole wheat flour + yeast, no. 2 for whole wheat flour alone, no. 3 for Nutrisoy R + yeast, no. 4 for yeast alone, no. 5 for extracted Nutrisoy R + yeast, no. 6 for Nutrisoy R + whole wheat flour (3: 1), no. 7 for Nutrisoy R + whole wheat flour (1: 1), and no. 8 for Nutrisoy R alone. The first seven curves are difficult to follow accurately in the reproduction but that does not matter because it is only the extremes (which can be followed) which show statistically significant differences.

ences are not statistically significant. However, irrespective of which percentage was used the mixture diets were much superior to Nutrisoy alone although somewhat inferior to whole wheat flour alone.

To check whether the fat content of Nutrisoy possibly contained a toxic component, the Soxhlet extract of Nutrisoy was added to an equivalent amount of whole wheat flour. Growth was not quite as good as on the whole wheat flour control but not greatly different either.[9]

However, after Soxhlet extraction, the Nutrisoy itself became completely unsuitable as food. As a matter of fact, most of the larvæ put on this diet died within a few weeks without any sign of growth. A few larvæ lived for four to five months but the largest size attained corresponded only to second or third or at

[9] Actually our first test of whole wheat flour plus soy extract resulted in high mortality and poor growth. Perhaps the solvent was inadequately removed; at least we were not able to repeat this result.

most fourth instar larvæ grown on whole wheat flour, although they may perhaps have molted a larger number of times (Mickel & Standish 1946). A single exception is recorded in the table: one dwarf pupa was obtained after 106 days when the flour had become lumpy. Presumably uncontrolled microorganisms could be responsible for supplying small amounts of growth factors.

Addition of Nutrisoy extract to extracted Nutrisoy gave results roughly comparable to those obtained from Nutrisoy itself.

Addition of yeast to either the extracted or the untreated Nutrisoy improved the nutritional value of the original diets greatly, so much so that the diets became practically as good as whole wheat flour although never as good as whole wheat flour plus yeast. It will be noted that yeast alone proved to be a good food; it was about as good as whole wheat flour.

The above results, compared with the known nutritional requirements of *T. confusum* (Fraenkel & Blewett 1942–1947) suggested that sterol and B vitamins, among other things, might have been responsible for the deficiency of the Nutrisoy flour. Experiments recorded in Table 1 and Figure 2 substantiated this idea although the addition of these known chemicals did not make the Nutrisoy diets approximate the controls.

When B vitamins were added to the Nutrisoy, the mixture produced significantly faster growth and heavier pupæ but no significant difference in percentage of pupation. The effect of the addition of cholesterol was more pronounced; when added to extracted Nutrisoy it not only restored the latter's food value but significantly increased the growth rate, pupal weight and percentage of pupation.

When both B vitamins and cholesterol were added to the Nutrisoy, the results showed no significant difference as to whether the Nutrisoy had been extracted or not; in both cases the food value of the Nutrisoy had been considerably improved. The addition of B vitamins does result in statistically significant differences from the addition of sterol alone (Table 1 and Figure 2) but explanation of the fact that statistically significant differences are shown in some cases by pupal weight and per cent pupation and in other cases by larval duration requires further study. In another test, not included in the graphs, the addition

of glucose to the Nutrisoy + B vitamin + sterol diet did not result in producing any significant differences from the control.

In spite of the addition of cholesterol and B vitamins, the Nutrisoy remained far short of the efficiency of whole wheat flour plus yeast in terms of pupal weight and growth rate. Obviously yeast and whole wheat flour must add something else that favors growth when mixed with Nutrisoy.

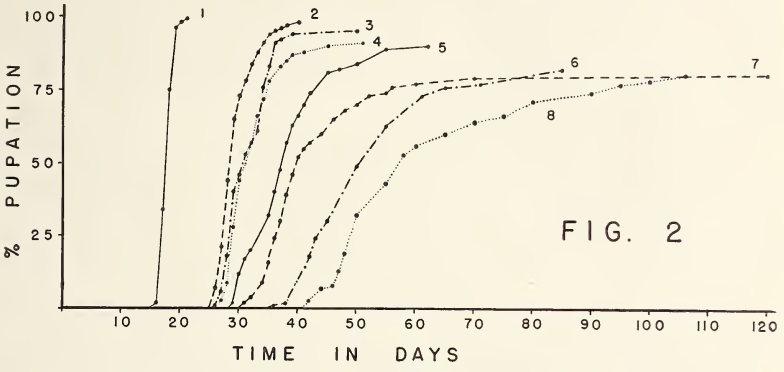

FIG. 2. Growth curves for larvæ of *Tribolium confusum* reared on Nutrisoy R flour with various supplements. Each curve based on data from 100 individuals. Curve no. 1 is for whole wheat flour + yeast, no. 2 for Nutrisoy R + cholesterol + B vitamins (see footnote no. 8), no. 3 for extracted Nutrisoy R + cholesterol + B vitamins, no. 4 for Nutrisoy R + cholesterol, no. 5 for extracted Nutrisoy R + cholesterol, no. 6 for Nutrisoy R alone, no. 7 for Nutrisoy R + B vitamins, and no. 8 for autoclaved Nutrisoy R alone.

In view of the fact that utilization of soybean protein by some vertebrates has been reported to be improved by autoclaving the soybean flour (Liener *et al.* 1949), the same treatment was tried here. However, the treatment proved to have no effect on pupal weight and per cent pupation while the larval duration on autoclaved Nutrisoy was found to be significantly longer.

DISCUSSION

When all the food preparations are compared[10] on the basis of pupal weight, percentage of pupation and larval duration, it will

[10] Data from experiments represented in Figure 1 and Figure 2 are not strictly comparable, since the two experiments were preformed at different times. Nevertheless, because the results for whole wheat flour plus yeast and for Nutrisoy from the two sets of experiments are very close, they are assumed to be adequately comparable for purposes of this discussion.

be noted that they can be divided roughly into three groups. On the favorable end are the whole wheat flour, yeast, and preparations of Nutrisoy that contained yeast, with whole wheat flour plus yeast always best. On the unfavorable end are Nutrisoy flour, autoclaved Nutrisoy flour, and extracted Nutrisoy flour, the last being entirely inadequate. In between are the mixtures whole wheat flour plus Nutrisoy flour, Nutrisoy flour plus cholesterol, Nutrisoy flour plus B vitamins, and Nutrisoy flour plus cholesterol plus B vitamins. It is interesting to note that cholesterol was more effective than the B vitamins but that the combination of the two was clearly best.

Obviously, Nutrisoy flour is not as good a food for the confused flour beetle as whole wheat flour. However, it is not entirely inadequate for the insects under the reported conditions, because approximately 80 per cent still developed to pupation although at a much slower rate. Some of the pupæ were spared to continue development and adults emerged (no observations were made to determine their reproductive capacity). From the results, it seems that the Nutrisoy flour used in these experiments was more favorable to the insect than the soy flours and soy grits used by Mickel & Standish (1946), which, they observed, "are not foods in which this insect can develop normally." However, the conditions under which the insects were reared and the food materials were not identical, therefore no direct comparison of results can be made.

In general, soybeans are high in protein, fat, and ash content but relatively low in carbohydrates (Markley & Goss 1944, Hafner 1942, Liener *et al.* 1949). Apparently, the high protein and low carbohydrate content would not explain the inferiority of the Nutrisoy flour, because Fraenkel & Blewett (1943c) have shown that absence of carbohydrate in the diet induced no increased mortality in this species and that the growth rate, though low, was still satisfactory.[11]

The vitamin requirements of the species have been worked out by Fraenkel & Blewett (1942–1947). *Tribolium confusum* re-

[11] Also another test, not reported in detail, showed little difference when glucose was added to Nutrisoy plus sterol and B vitamins.

quires thiamin, riboflavin, niacin, pyridoxin, pantothenic acid, biotin, folic acid and a sterol; it may also need some choline, *p*-amino-benzoic acid, and inositol.

Soy beans are reported to be a good source for numerous vitamins including thiamin, niacin, pantothenic acid, biotin, riboflavin, and pyridoxin (See Markley 1950). We have not found any report on the presence of folic acid. The analysis supplied for our Nutrisoy sample agrees with the above at least for the components analyzed (Footnote No. 3), and also records the presence of choline. Perhaps only one of the B vitamins is concerned with the improvement in weight and growth rate resulting from the addition of the B vitamin complex.

Deficiency of sterol was found to be responsible to a fairly large extent for the deficiency of Nutrisoy flour as food to *T. confusum*. The oil of soybeans is reported to contain about 0.4 per cent sterols (stigmasterol, sitosterols and dihydrositosterol) (Markley & Goss 1944), but the Nutrisoy flour used in the present study is a "defatted" product (0.96 per cent fat; = ca 5 per cent of original) which, to judge from our data, must contain some utilizable sterol but not enough for *T. confusum*. Although the sterol content of yeast is not very high (0.5–0.75 per cent sterols and ergosterols according to the manufacturers), apparently it did supply enough so that there was practically no difference whether the Nutrisoy flour to which the yeast was added had been extracted or not.

When Nutrisoy flour was mixed with whole wheat flour, there seemed to be some unfavorable effect of the mixture to the insect, although in vertebrate nutrition the proteins of wheat and soybean are said to be supplementary (Hove, Carpenter & Harrel 1945, Sherman 1946). Compared to the mixtures, the whole wheat flour was significantly superior in terms of pupal weight and larval duration, although there was no significant difference in pupation percentage. Such differences must be due to some factor in the soy flour other than could be explained by the lower concentration of the whole wheat flour, unless one assumes that the larvæ could and did select soy flour particles from the mixture; this seems highly unlikely. As discussed before, the fact that whole wheat flour plus yeast was significantly superior to

Nutrisoy flour plus yeast and Nutrisoy flour plus cholesterol plus B vitamins suggests that the Nutrisoy flour was in some respects inadequate. In vertebrate nutrition studies, raw soybean meal has been found to contain trypsin inhibitors which have a deleterious effect on the growth of experimental animals (Ham & Sandstedt 1944, Ham, Sandstedt & Mussehl 1945). Liener *et al.* (1949), using soy flour of the same trade name (Nutrisoy XXX) to feed rats, showed that diets containing autoclaved (20 minutes at 15 lbs. pressure) soybean meal consistently supported better growth than the unheated meal, the heat treatment therefore inactivated these inhibitors. In our experiment, however, no such improvement was obtained.

It is possible that the soy protein is inadequate for the insect, but since we do not know the amino acid requirements of the insect or the amino acid composition of the Nutrisoy flour, there is no basis for speculation. While the data do not eliminate the possibility that soy flour may contain some inhibitory component, it will be recalled that addition of dried yeast to the Nutrisoy flour made it adequate and that the addition of whole wheat flour also gave favorable growth, while the addition of B vitamins and sterol gave improved but slow growth (also small size). We can interpret this as the yeast having supplied B vitamins, sterols and something else to the soy flour; the additional factor may have been a protein supplementing the soy protein.

SUMMARY

In rearing tests at 30° C. and 70 per cent R.H. with Nutrisoy R, a defatted soybean flour, it was found that:

1. The Nutrisoy flour used is not a good food for *Tribolium confusum* larvæ when compared with whole wheat flour but about 80 per cent pupation can be obtained if the experiment is continued for three months.

2. After extraction with an ether-alcohol mixture the Nutrisoy flour was rendered entirely unsuitable for the growth or even maintenance of life of *T. confusum*.

3. The food value of Nutrisoy flour for *T. confusum* was nearly equal to the controls when dried yeast was added. This was true irrespective of whether or not the flour had been ex-

haustively extracted with an ether-alcohol mixture. Whole wheat flour was also effective but less so than yeast.

4. Addition of the extract of the Nutrisoy flour to the extracted soy flour produced nearly the original food value for *T. confusum* again. Addition of cholesterol greatly enhanced the value of both extracted and normal Nutrisoy flour. Addition of pure B vitamins also improved the food value of the Nutrisoy flour considerably, but much less so than the addition of cholesterol. Addition of glucose was without significant effect.

5. The deficiency of sterol and B vitamins, however, can not wholly account for the deficiency of Nutrisoy flour as a food for this beetle.

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ASSOCIATION FOR THE STUDY OF ANIMAL BEHAVIOUR

Recently, the editor received a copy of The Bulletin of Animal Behaviour, No. 9, March, 1951, publication of the Association (founded March 13, 1936). The bulletin contains three Karl Von Frisch papers on Recent Advances in the Study of the Orientation of the Honey Bee, a paper by W. H. Thorpe on Animal Behaviour Terminology, a paper by O. Koehler on The Ability of Birds to Count and one by E. A. Armstrong on The Nature and Function of Animal Mimesis. This bulletin and the previous eight numbers cover a wide variety of behavior topics. Nonmembers of the Association may purchase copies at the price of 7 s. 6 d. and obtain information on membership by addressing the Hon. Secretary, Alastair N. Worden, Cromwell House, Huntingdon, England.—F. A. S.